

# Monitoring individual human cells during exposure to environmental organic toxins: Synchrotron FTIR spectromicroscopy

Hoi-Ying N. Holman<sup>a</sup>, Miqin Zhang<sup>b</sup>, Regine Goth-Goldstein<sup>a</sup>, Michael C. Martin<sup>c</sup>,  
Marion Russell<sup>a</sup>, Wayne R. McKinney<sup>c</sup>, Mauro Ferrari<sup>b,d</sup>, and Jennie C. Hunter-Cevera<sup>a</sup>

<sup>a</sup> Center for Environmental Biotechnology, Lawrence Berkeley National Laboratory, Berkeley, CA 94720

<sup>b</sup> Dept. of Material Sciences, University of California, Berkeley, CA 94720

<sup>c</sup> Advanced Light Source Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720

<sup>d</sup> Biomedical Engineering Center, Ohio State University, Columbus, OH 43210-1002

## 1. INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) and organochlorines (OCs) are ubiquitous environmental toxins that are known rodent carcinogens and suspected human carcinogens. Human exposure to these compounds is of great concern and needs to be assessed. Traditional assessment of human exposure and health risk to these toxins relies primarily on animal experiments. A major uncertainty inherent to this approach is the extrapolation from the high-dose animal experiments to the low-dose and long-term human exposure. To overcome this uncertainty, a common strategy is to complement animal experiments with short-term assays that use human cell culture systems and biomarkers that are indicative of exposure. The cytochrome P4501A1, encoded by the gene *CYP1A1*, metabolizes PAHs. *CYP1A1* levels increase as a result of exposure to PAHs or OCs through binding of these compounds to the Ah receptors [1]. Thus the induction of the *CYP1A1* gene expression and/or increase in the associated enzyme activity are selected as global biomarkers for PAH and OC exposure. The increase in *CYP1A1* transcript levels correlates with the levels of the exposure, which can be quantified by reverse transcription-polymerase chain reaction (RT-PCR) [2].

In this study we are developing a faster (than current RT-PCR techniques) and still sensitive method for detecting the exposure of individual human cells to PAHs and OCs. The development involves two key components. First, we identify suitable signals that represent intracellular changes that are specific to PAH and OC exposure. Second, we will construct a device to so guide the biological cell growth that suitable signals from individual cells are consistent and reproducible for a given set of exposure conditions. This paper describes our use of synchrotron-radiation-based (SR) Fourier-transform infrared (FTIR) spectromicroscopy in the mid-IR region (4000-400  $\text{cm}^{-1}$ ) as a tool to identify IR radiation signals (from individual cells) that are specific to the intracellular response after their exposure to PAHs or OCs. SR FTIR spectromicroscopy was used because it has been proven to be a sensitive analytical technique capable of providing molecular information in a biological system quickly (within minutes) at dilute concentrations and a spatial resolution of 10 microns [3-4]. The potential use of the new method to screen for the exposure to PAHs and OCs at environmentally relevant concentrations was tested using human cells in an environmental medium for which contaminant types and levels are known. In these tests, HepG2 (human hepatoma derived) cells modeled the human epithelial cells that would activate the test compound PAHs and OCs; benzo(a)pyrene (BaP) modeled the PAHs, and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) modeled the OCs. HepG2 cells metabolize PAHs efficiently. They also show *CYP1A1* induction after their treatment with PAHs and OCs. The potential use of our marker signals for detecting intracellular response to mixtures of PAHs was also demonstrated in an

environmental medium with dilute concentrations of coal tar, a complex mixtures of different PAHs.

## 2. EXPERIMENTAL

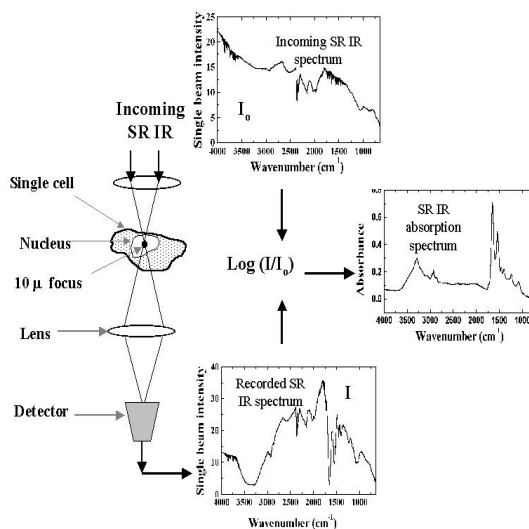
The infrared spectromicroscope in conjunction with Beamline 1.4.3 at the Advanced Light Source (ALS) at Lawrence Berkeley National Laboratory was used to monitor intracellular changes in response to TCDD, BaP, and coal tars exposure. Figure 1 depicts the experimental setup for this direct measurement.

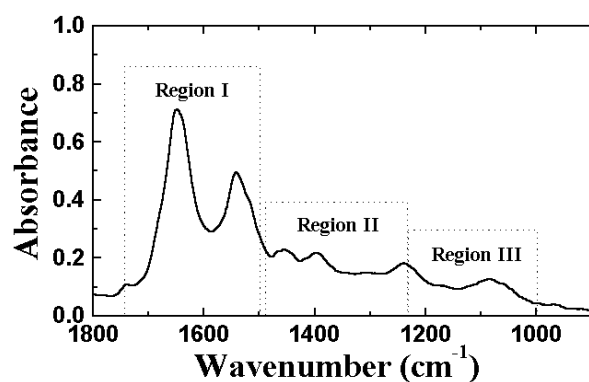
All SR FTIR spectra were recorded in the 4000–650  $\text{cm}^{-1}$  infrared region. This region was selected because it is the region that contains unique molecular fingerprint-exhibiting absorption features of intact biomolecules. For each IR measurement, 128 spectra were co-added at a spectral resolution of 4  $\text{cm}^{-1}$ . All spectra were obtained in the reflection geometry and were ratioed to the reflectance spectrum of a gold-coated slide to produce absorbance values (see Figure 1).

**Figure 1.** Diagram illustrating experimental setup for monitoring intracellular response to polycyclic aromatic hydrocarbons (PAHs) and organochlorine (OCs) exposure. (Note that cells were placed on treated surfaces on a microscope stage. All measurements were made in reflectance mode.)

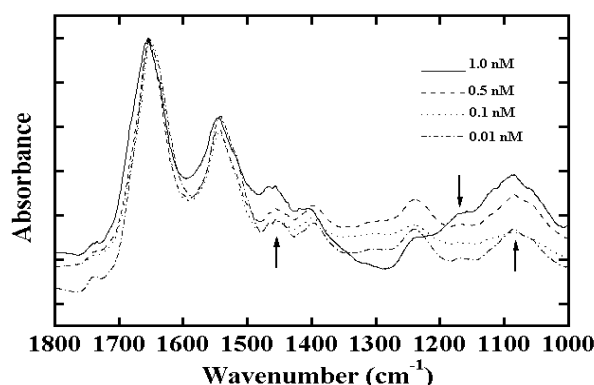
## 3. RESULTS AND DISCUSSION

We obtained SR FTIR absorption spectra in the range (1750–1000  $\text{cm}^{-1}$ ) of interest at different locations of a randomly selected single HepG2 cell prior to its exposure to the test chemicals. Figure 2 shows a typical absorption spectrum recorded at the proximity of a cell nucleus. The overall spectral characteristics are similar to those reported in [6]. It shows three main informative spectral regions for the architectural details of nucleic acid helices; each corresponds to a molecular subgroup [6]. Absorption bands in Region I ( $\sim 1750\text{--}1500\text{ cm}^{-1}$ ) arise from in-plane vibrations of the bases (in addition to other proteins), and are sensitive to base-pairing and base-stacking effects. Absorption bands in Region II ( $\sim 1490\text{--}1230\text{ cm}^{-1}$ ) arise from base deformation motions coupled through the glycosidic linkage to sugar vibrations. Their spectral characteristics are strongly dependent on the glycosidic torsion angle. Absorption bands in Region III ( $\sim 1230\text{--}1000\text{ cm}^{-1}$ ) arise from phosphate vibrations and sugar vibrations. The absorption band positions and intensities depend strongly on the base-base interactions and the helical geometry in nucleic acids. The architectural structures of nucleic acid helices are extremely sensitive to the surrounding environments. We anticipate that exposing individual cells to the test chemicals, at the appropriate dose and exposure time, will lead to changes in the SR FTIR spectral characteristics in the three regions.





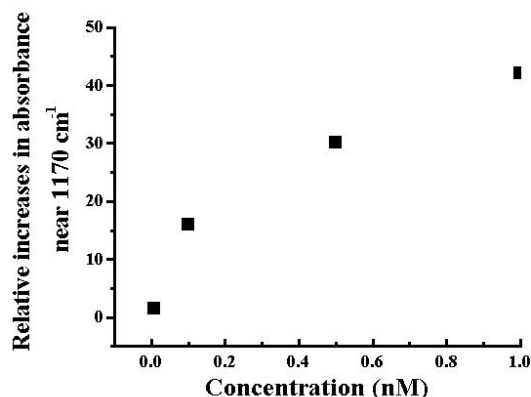
**Figure 2.** A typical SR FTIR absorption spectrum recorded near/at the nucleus of a single HepG2 cell prior to its exposure to the test chemicals. The spectrum shows three main informative spectral regions. Regions I-III are discussed in text.

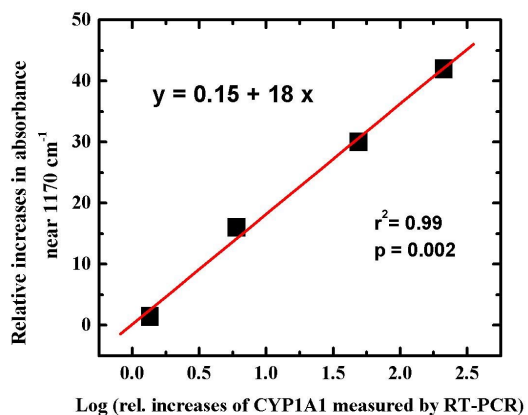


**Figure 3.** Normalized absorbance spectra of individual HepG2 cells after 20-hour exposure to different TCDD concentrations.

To identify spectral signals that were specific to the induction of *CYP1A1*, cells were exposed to TCDD known to interact specifically with the Ah receptor that regulates *CYP1A1* expression. HepG2 cells were treated with different concentrations of TCDD (0, 0.01, 0.1, 0.5, and 1.0 nM) for 20 hours. SR FTIR spectra were measured for randomly selected individual cells, and examined for significant changes in the spectral characteristics as the dose increased. Figure 3 shows the normalized SR FTIR absorption spectra recorded. There are considerable differences in the SR FTIR spectra, with one difference being the increased absorption of the vibration band 1180 - 1160  $\text{cm}^{-1}$ , centered at  $\sim 1170 \text{ cm}^{-1}$ . This systematic spectral change might be related to the alteration in the DNA base structure [7]. This will be the subject of future investigation. For our experimental system, the normalized absorbance intensity for individual cells increased from 0.007 to 0.21 when the TCDD concentration increased from  $10^{-11}$  to  $10^{-9} \text{ M}$  (Figure 4). The normalized absorbance intensity at  $\sim 1170 \text{ cm}^{-1}$  for individual control cells was 0.005. This is a 42-fold increase in the absorbance intensity, which is extremely significant considering the definition of absorbance (see Figure 1).

**Figure 4.** Dose response in absorption intensity at about  $1170 \text{ cm}^{-1}$ .





**Figure 5.** SR FTIR spectromicroscopy versus RT-PCR techniques.

We compared the performance of SR FTIR spectromicroscopy with our current RT-PCR technique. *CYP1A1* expression in HepG2 cells, as measured by RT-PCR, increased 212-fold over the same range of TCDD concentrations used in the SR FTIR study. Relative changes in the absorption intensity at  $\sim 1170\text{ cm}^{-1}$  (detected by SR FTIR spectromicroscopy) as compared to the *CYP1A1* expression detected by the RT-PCR techniques are shown in Figure 5. The solid line in Figure 5 is the

least-squares fit to the data. The excellent agreement (with  $r^2 = 0.99$ ) for measurements from the two methods indicates that the fast SR FTIR spectromicroscopy technique is indeed comparable to the RT-PCR technique that specifically measures increases in the *CYP1A1* expression. This demonstrates that SR FTIR spectromicroscopy can be used as a substitute for direct measurements of *CYP1A1* levels.

#### 4. CONCLUSIONS

We have succeeded in using SR FTIR spectromicroscopy to identify specific that represent the intracellular response to PAH and OC exposure. Further development will allow the technique to distinguish biological responses to other different environmental toxins.

#### ACKNOWLEDGEMENTS

This work was performed with support by the Directors, Office of Energy Research, Office of National Petroleum Technology Program, Offices of Health and Environmental Sciences, Biological and Environmental Research Program and Basic Energy Sciences, Materials Science Division, of the United States Department of Energy under Contract No. DE-AC03-76SF00098.

#### REFERENCES

1. S. Safe, "Modulation of gene expression and endocrine response pathways by 2,3,7,8,-tetrachlorodibenzo-*p*-dioxin and related compounds", *Pharmac. Ther.* **67**, pp. 247-281, 1995.
2. M. Russell, J. Marshall-Scott, R. Goth-Goldstein, " Biomarker of exposure for polycyclic aromatic hydrocarbons", manuscript in preparation, 1999.
3. H.-Y. N. Holman, D.L. Perry, M.C. Martin, W.R. McKinney, "Applications of synchrotron infrared microspectroscopy to the study of inorganic-organic interactions at the bacterial-mineral interface," *Application of Synchrotron Radiation Techniques to Material Science IV*, S.M. Mini, S.R. Stock, D.L. Perry, and L.J. Terminello (eds.), **524**, pp. 17-23.
4. N. Jamin, P. Dumas, J. Moncuit, W-H. Fridman, J.-L. Teillaud, G.L. Carr, G.P. Williams, "Highly resolved chemical imaging of living cells by using synchrotron infrared microspectrometry," *Proc. Natl. Acad. Sci., USA*, **95**, pp. 4837-4840, 1998.
5. M.C. Martin, W.R. McKinney, " The first synchrotron infrared beamlines at the Advanced Light Source: microspectroscopy and fast timing", *Application of Synchrotron Radiation Techniques to Material Science IV*, S.M. Mini, S.R. Stock, D.L. Perry, and L.J. Terminello (eds.), **524**, pp. 11-15.
6. J. Liquier, E. Taillandier, "Infrared spectroscopy of nucleic acids", *Infrared Spectroscopy of Biomolecules*, H. H. Mantsch, D. Chapman (eds.), **pp.** 131-158, Wiley-Liss, New York, 1996.
7. D.C. Malins, N.L. Polissar, K. Nishikida, E.H. Holmes, H.S. Gardner, S.J. Gunselman, "The etiology and prediction of breast cancer - Fourier transform-infrared spectroscopy reveals progressive alterations in breast DNA leading to a cancer-like phenotype in a high proportion of normal women," *Cancer*, **75**, pp.503-517, 1994.

Principal investigator: Hoi-Ying Holman, Earth Sciences Division, Ernest Orlando Lawrence Berkeley National Laboratory. Email: HYHolman@LBL.gov. Telephone: 510-486-5943.